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Culture of the California Red Abalone *Haliotis rufescens* Swainson (1822) in Chile

by

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Abstract. *Haliotis rufescens* has been experimentally cultured and maintained during hatchery research in Coquimbo, Chile. Spontaneous spawnings in laboratory populations were observed in spring months from 1979 to 1982, accompanying temperature changes of the ambient seawater. Spawning was artificially induced at various times of the year without conditioning. Broods were produced from both spontaneously occurring and induced spawnings. Once capable of feeding on macroalgae, abalone were reared on the locally abundant *Lessonia flavicans,* although it was experimentally shown that young juveniles grew best when presented with a mixed diet of macro- and microalgae.

INTRODUCTION

THE EXTENSIVE rocky Chilean sublittoral environment, with its cool temperature and rather extensive macroalgae assemblages appears to offer suitable habitat for the growth of abalone, *Haliotis* spp. However, endemic haliotids are unknown in Chile although many other coastlines of the world with similar characteristics host several species of this genus (COX, 1962). The zoogeographical problem of why no haliotids occur in Chile and the possibility of introducing *Haliotis* spp. to Chile as a new marine resource have long interested both Chilean and U.S. researchers. A one-year study, supported by the Organization of American States (OAS), examined the feasibility of bringing an abalone species from California to Chile under quarantined test conditions (EBERT, 1980). In September 1979, about 300 adult and juvenile hatchery-reared *Haliotis rufescens* were brought from California to Chile by two of us (Owen and Ebert), and maintained at the Department of Marine Research (DIMAR), University del Norte, Coquimbo (29°59′S, 71°22′W). Laboratory and field experiments were conducted to examine ecological relationships between the red abalone and endemic Chilean species. Inclusive were studies to determine predator-prey relationships and adequacy of the macroalgae (acceptance, food conversion efficiency, and abalone growth rates). Early in this study some abalone spawned spontaneously; the resulting larvae were reared under improvised conditions. In this report, we document results of this culture and of a later culture from a spawning induced by the methods of KIKUCHI & UKI (1974). We also include data on the comparative growth of small juveniles raised on different diets of Chilean algae.

MATERIALS AND METHODS

DIMAR is located on the northeast margin of Herradura Bay, which is a semi-protected coastal embayment with an oceanic salinity regime. The bay receives negligible freshwater input and sources of contamination are minimal. The temperature range at 10-m depth is typically 13–15°C (ALFSEN, 1979).

Spontaneous Spawning

Several adult abalone, 12 cm in length, which had been in Chile for two weeks, spawned unexpectedly on 22 September 1979 within tanks of continuously renewed seawater at a temperature of 14.4°C. Seawater used as the culture medium for these gametes, larvae, and juveniles was obtained directly from an intake at 10-m depth on the bay bottom. This water was filtered in the laboratory using polypropylene filter bags (GAF Corp.) which retained particles greater than 10 μm. About 3 million eggs from one female were fertilized with about 10 mL of a dense sperm suspension from two males, and the progress of fertilization was monitored by microscopy. After one

hour, fertilized eggs were rinsed to remove excess sperm by three decantations following fresh seawater refills, and were distributed evenly, as judged by sight, to one layer on the plastic pail bottom. Each pail contained 10 L of seawater at 12.5–13.5°C. The static seawater in these cultures rose to air temperature of 17°C over 12 h. Upon reaching the veliger stage, abalone larvae were gently rinsed onto a 52-μm mesh nylon screen (Nytex; Tetko, Inc., Elmsford, New York), and distributed at several different densities into plastic containers of 10, 20, or 100-L capacity. These culture containers were subject to ambient temperature fluctuations between 13 and 18°C. All seawater was changed daily in the smaller cultures, and one-third the volume was changed daily in the 100-L tanks. Growth and development of larvae were observed daily and mortality was estimated from the quantity of empty shells recovered by screening during seawater changes. Microalgae that settled and grew on container surfaces provided forage. Newly settling larvae were given pure cultures of *Tetraselmis suecica,* and as early growth of post-larvae progressed, pennate diatoms (unidentified) harvested from aquarium surfaces in the laboratory were introduced to the cultures as food.

At a mean size of approximately 5 mm, the abalone juveniles were transferred to 1000-L rectangular fiberglass tanks which received a continuous flow of unfiltered seawater from 10-m depth in the bay. At this time, pieces of *Lessonia flavicans, L. nigrescens,* and *Macrocystis integrifolia* were offered to the juvenile abalone, although feeding with microalgae was continued until the young abalone were fully capable of consuming the macroalgae.

Induced Spawning

Abalone were induced to spawn in April 1980 and a controlled rearing experiment was carried out over 12 months. Fertilization and larval rearing were carried out in a heated laboratory at 18–19°C. Seawater was pumped to this laboratory through an offshore sand filter located at 3-m depth on the bay floor. This water was filtered to 10 μm in the laboratory, and normally had a temperature of 15°C. It was held without further treatment and, after reaching the laboratory temperature of 18°C, was used for the handling of gametes, larvae, and small juveniles.

Two male and two female abalone (6–8 cm in length) that appeared ripe were transferred from holding tanks and placed separately by sex into 20-L plastic pails. These pails were supplied with running seawater at 14°C, fitted with screens on top to prevent crawling out, and left overnight. On the following morning, fecal material was washed from the pails, and the abalone were given a seawater flow of 250 mL/min at 17°C via an ultraviolet (UV) water treatment unit (REFCO Intl., Hayward, CA, model RL-10). The seawater temperature was allowed to rise to 18°C over a 3-h period as treatment proceeded. In a subsequent spawning in September 1980, the same methodology was used, with overnight running seawater at 13°C and with the temperature of UV irradiated seawater rising from 14 to 18°C over a 3-h period. In both spawnings about 10^6 eggs were retained in 10 L and treated as in the spontaneous spawning. About 25,000 veligers were introduced into each of two 100-L cylindrical fiberglass tanks (3 individuals/cm^2 of wetted tank surface area) and the rest were discarded. One-third of the water was changed daily in these tanks and a complete rinse was done every 10 days.

Settled abalone were given pure cultures of *Tetraselmis suecica* as required during the first three weeks of culture. After this time, film-forming pennate diatoms were added to the cultures. When juveniles reached an average size of 1.8 mm, they were transferred to seawater trays in a greenhouse. The abalone fed on naturally occurring diatoms that grew in the trays, and upon reaching 5 mm they were transferred to a 1000-L fiberglass tank at a density of about one per 500 cm^2. There they were maintained on *Lessonia flavicans.*

A spawning was induced in September 1980 to duplicate the April 1980 observations; in this test, only early developmental events were observed. Further spawnings were made during 1980 and 1981 to check on the seasonal condition of abalone brought from California and to note inception of sexual maturity in cultured abalone.

Forage Experiment

Observations of feeding on macroalgae were made to determine which of three Laminariales combined with microalgal films promoted the best growth of juvenile abalone. Five groups of 20 abalone having an average size of 11.2 mm (range = 11.0–11.5 mm) were distributed between five 20-L aquaria receiving a continuous flow of sand-filtered seawater at temperatures of 13–17.5°C.

All abalone were given microalgal films cultured on 15 × 20 cm plastic sheets in the following combinations with species of Laminariales: (Diet A) none, (Diet B) *Lessonia flavicans,* (Diet C) *L. nigrescens,* (Diet D) *Macrocystis integrifolia,* and (Diet E) all three spp. of macroalgae. About 30 g fresh weight of macroalgae pieces were added to each aquarium along with an algae-covered plastic sheet. Microalgal films consisted mainly of pennate benthic diatoms with scattered bluegreen algal filaments and green algal cells. The abalone always had an abundance of food upon which to graze; food was changed routinely to ensure freshness, and fecal matter and debris were siphoned off every other day.

The experiment was run for 75 days, and shell length was measured every 15 days using a millimeter rule, estimating to the nearest 0.25 mm. Abalone were handled using a 10 mm camel's hair brush to avoid damaging their delicate shells. Differences between growth on the different diets were tested for significance using a Model I analysis of variance and a Student-Newman-Keuls (SNK) test (Sokal & Rohlf, 1969).

Table 1

Parameters of early development of *Haliotis rufescens* obtained in three spawnings in Chile, with comparative values from the literature. h, hours; d, days.

Parameter	Spawnings in Chile: Spontaneous 22 Sept 79	Spawnings in Chile: Induced 23 Apr 80	Spawnings in Chile: Induced* 9 Sept 80	Leighton (1974)	Ebert & Houk (1984)
Blastula (h)	—	5	5	5	5
Rotating gastrula (h)	16	—	10–12	13	15
Trochophore emergence (h)	22–24	16	16–24	18–24	20
Veliger stage (h)	40	24	29	48	30
Surface tactile stage (d)	5–8	4	3	4	6
Settlement (d)	8–12	4–7	3–7	5–6	7
Notch stage (d)	45	35–40	—	60–70	50
Temperature (°C)	13–18	18	17–19	14–18	15

* Not cultured post-metamorphosis.

RESULTS

Culture Experiments

Spawning: The spontaneous spawning of 22 September 1979 was probably due to one of the small though abrupt rises in water temperature that occur irregularly in waters near Coquimbo during spring and summer months (Alfsen, 1979). Abalone from the same group spawned spontaneously in their holding tanks on 9 October 1980 when the temperature of incoming water rose overnight from 14.2 to 15.5°C. The phenomenon occurred again on 24 October 1981 when ambient seawater temperature rose from 14.8 to 16°C overnight. In these cases, the female abalone crawled from the holding tanks while simultaneously ejecting ova; males remained below the water surface while releasing sperm.

Abalone were successfully spawned in April, September, and November 1980, and in June, August, and October 1981. In all cases, the abalone spawned within 2 to 4 h after exposure to UV-treated seawater. The spawning of October 1981 included both abalone from California which had been immature when brought to Chile, and abalone cultured in Chile which had reached two years of age. Three-year-old California abalone spawned copiously and produced normal larvae. Of the two-year-old abalone produced in Chile, only 20% exhibited pigmented gonadal tissue. They spawned but produced few eggs, and these failed to be fertilized; no further observations were made on development of sexual maturity in these abalone.

In all spawnings producing viable zygotes, fertilization and early development proceeded in accordance with descriptions in the literature. Some parameters of early development in three cultures are listed in Table 1.

Survivorship and growth: Mortality was negligible in larvae cultures. After the larvae had metamorphosed, a steady attrition of postlarvae began until they neared the 2-mm size. Table 2 lists survivorship and size for the two groups of cultured abalone. In this table it can be seen that only 0.4% of the abalone in Group II reached the 1.8-mm size from the veliger. However, survival from the 1.8-mm to the 20-mm size neared 50% over the 12 months of observation. Abalone surviving to 35 months reached maximum sizes of 75 mm. All data past 12 months include fortuitous observations outside the original experimental design and represent survival and growth of abalone maintained under less than optimal conditions. Comparisons between the two groups of abalone show an almost equal survival rate when comparing the 6 to 12 month survival of Group I with the 4 to 12 month survival of Group II (Table 2).

Shell color of the cultured abalone was uniformly red and white "candy stripe" to a size of 2 mm. Past this size,

Table 2

Size and age of *Haliotis rufescens* cultured in laboratories at Coquimbo, Chile, and Monterey, California.

Group	Age (months)	Shell length, mm: Mean ± SD	Shell length, mm: Range	n
I*	6	— —	2.0–16.5	—
	12	22.4 ± 3	14.0–30.0	270
	24	34.5 ± 5	21.0–48.0	1785
	36	53.8 ± 6	37.0–75.0	150
II**	2	1.8 —	1.3–2.2	40
	4	5.9 ± 1	2.0–10.0	1637
	6	10.9 —	7.0–16.0	108
	12	20.1 ± 4	12.0–33.0	970
III***	2	1.8 —	1.2–2.3	50
	4	5.0 —	3.0–7.8	50
	6	8.1 —	4.5–12.4	50
	8	13.0 —	10.5–16.5	50
	12	20.5 —	12.2–26.1	50

* Spontaneous spawning; Coquimbo, Chile; Sept. 1979.
** Induced spawning; Coquimbo, Chile; April 1980.
*** Composite of several induced spawnings; Calif. Dept. of Fish and Game, Monterey, California.

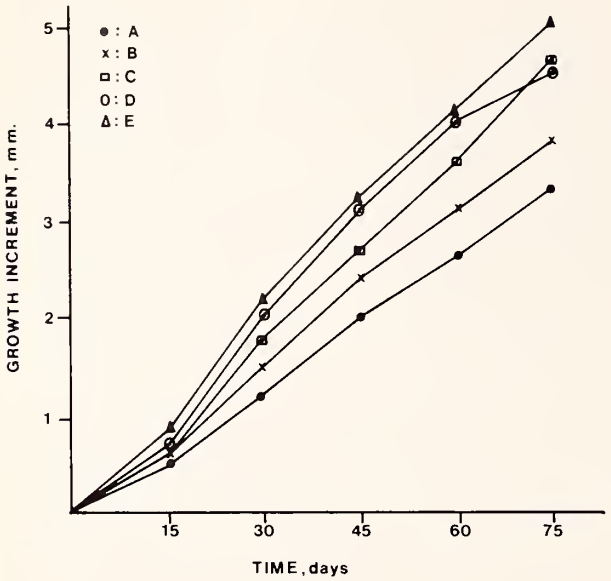

Figure 1

Growth of juvenile abalone on five different algal diets. Each data point represents the mean growth increment for all animals in each treatment. Diets: A = microalgae (ma); B = *Lessonia flavicans* plus (ma); C = *L. nigrescens* plus (ma); D = *Macrocystis integrifolia* plus (ma); E = all macroalgae plus (ma).

a complex variation in color developed among the specimens, including white, green, and red pigment, solidly or in bands. As long as they were provided fresh seawater, forage, and adequate physical space, the abalone appeared hardy and free of disease. Growth was retarded in a group of 5–10 mm abalone that were crowded in a holding tank at a density of 1–2 per 100 cm^2. In spite of this crowding, the abalone never showed a tendency to crawl out of the tanks. They resumed normal growth when presented adequate physical space.

Feeding experiment: The diet of microalgae alone produced the least growth. Different species of macroalgae gave different growth rates when in combination with microalgae, and a mixture of all the macroalgae plus microalgae produced optimal growth (Figure 1, Table 3). Statistically significant ($P = 0.01$) differences between groups appeared beginning at 30 days and continued through the end of the experiment (Figure 1, Table 3).

DISCUSSION

Breeder and juvenile red abalone from California acclimatized well to ambient seawater conditions at the Coquimbo laboratory. Laboratory tests performed during 1979 and 1980 revealed that the abalone readily accepted native algal species such as *Lessonia flavicans, L. nigrescens, Durvillea antarctica,* and *Gracilaria* sp. (EBERT, 1979a, b, 1980). Abalone growth, feeding rates, and food-conversion efficiency tests were performed using *L. flavicans*. Juvenile red abalone fed this species grew an average of 10.5 mm in shell length (16.5–27.0 mm) during a 7-month period (EBERT, 1980). This compares favorably with juvenile red abalone growth rates observed in California when giant kelp, *Macrocystis* spp., a preferred food for adults, is fed. The juvenile red abalone feeding rate and food-conversion efficiency on *L. flavicans* during the same 7-month period noted above averaged 7.57% and 9.36% of their body weight/day respectively (EBERT, 1980). Our spawnings showed that at least a portion of the group was ripe throughout the year, suggesting that these animals were fecund the year around in Chilean water, as shown for this species in its California habitat (*e.g.,* BOOLOOTIAN *et al.,* 1962).

Larvae produced in our first culture settled in 8–12 days, and those of the second and third cultures in 3–7 days (Table 1). The longer delay of the first culture was unusual and may indicate stress due to temperature variation under the improvised conditions available at the time. In subsequent cultures where water temperatures were stable, settling time was comparable to literature values. Early abalone development rates in Chile were similar to those observed in California, although a direct comparison is somewhat obscured by the variable culture temperatures within and among the various studies (Table 1).

No mass mortalities were ever experienced at any stage

Table 3

Mean sizes of abalone for which incremental growth data are plotted in Figure 1. At time zero, for all abalone $\bar{x} = 11.2$, $s = 2.5$, $n = 20$. N, total number per treatment; $\bar{x}$, mean length; s, standard deviation.

	Time, days														
	15			30			45			60			75		
Diet[1]	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n	$\bar{x}$	s	n
A	11.7	0.34	20	12.4	0.40	20	13.2	0.55	20	13.8	0.47	20	14.5	0.64	20
B	11.8	0.29	20	12.7	0.41	20	13.6	0.52	20	14.3	0.52	20	15.0	0.72	20
C	11.8	0.29	20	13.0	0.43	20	13.9	0.55	20	14.8	0.57	20	15.8	0.60	20
D	11.9	0.22	20	13.2	0.41	20	14.3	0.50	20	15.2	0.66	20	15.7	0.68	18
E	12.0	0.29	19	13.4	0.41	19	14.4	0.44	19	15.3	0.66	18	16.2	0.83	18

[1] See Figure 1.

in our cultures in Chile, although the constant low level attrition of postlarvae produced an overall low survival to the 2-mm size. At the time the first respiratory pore appeared, mortality abated. A similar pattern is usually found in California abalone culture research and hatchery operations. California cultivators occasionally experience high postlarval mortalities, presumably from pathogenic bacteria. California hatchery operations also suffer high mortalities in early juvenile stages due to food depletion by copepods and depredation by nematodes and other small invertebrates. For example, the copepod *Tigriopus californicus* competes with young abalone for forage and space and degrades water quality. The role of nematodes is poorly understood, but they are commonly observed feeding on weakened and freshly dead abalones. None of these problems was apparent in Chile. Successful hatchery production of this abalone in Chile will rely in part on solution of the postlarval attrition problem.

Few abalone of any size were lost due to their crawling out of the tanks (often a serious problem in some California hatcheries), even at densities as high as one individual ($\bar{x}$ = 22 mm long) per 70 cm^2 tank space. At this density, microalgal films were rapidly eliminated, probably depriving the abalone of critical nutrition, thereby stunting their growth. SHIBUI (1972) found diatoms to be the optimum food for *Haliotis discus hannai* in the 3 to 10 mm size range.

Mean growth rates in Chilean produced *Haliotis rufescens* are similar to those observed in California, at least during the first year of life. A wide size range is apparent from all groups of similar age abalone (Table 2).

To our knowledge, this is the first experience of culturing a northern hemisphere haliotid in the southern hemisphere. These preliminary results suggest there are no reasons to preclude success in development of *Haliotis* cultures in Chile. The lower labor costs and present lack of legal restrictions may make this culture economically attractive in Chile in the future. The fate of red abalone introduced to the Chilean sublittoral has not been examined. Experimental plantings in suitable habitats have yet to be conducted. However, laboratory and field experimentation made during the OAS project (EBERT, 1980) gave indications that such transplants would be successful and ecologically compatible with the Chilean nearshore environment. However, further experimentation is recommended, with a male-only population, prior to any decision to introduce *H. rufescens* to natural Chilean marine environments.

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